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(57) Abstract

Homogentisic acid derivatives, pharmaceutical compositions comprising such homogentisic acid derivatives, and a method of treatment using such derivatives for the treatment of conditions wherein protein kinase C inhibition is therapeutically indicated. In particular, the present invention discloses the treatment of the following PKC-mediated disease states using homogentisic acid derivatives: cancer, cardiovascular and renal disorders, inflammation, central nervous system disorders, immunosuppression, and septic shock.

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HOMOGENTISIC ACID DERIVATIVES, METHODS OF TREATMENT OF DISEASE STATES MEDIATED BY PROTEIN KINASE C USING HOMOGENTISIC ACID DERIVATIVES, AND PHARMACEUTICAL COMPOSITIONS THEREOF

FIELD OF THE INVENTION

The present invention relates to the use of homogentisic acid derivatives for the treatment and prevention of conditions wherein protein kinase C inhibition is indicated. In particular, the present invention relates to the treatment of cancer, cardiovascular and renal disorders, inflammation, central nervous system disorders, immunosuppression and septic shock using homogentisic acid derivatives.

BACKGROUND OF THE INVENTION

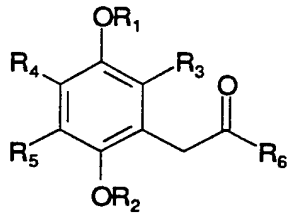
Protein kinase C (hereinafter "PKC") is a calcium and phospholipid activated enzyme that plays a significant role in mediating the effects of a host of hormones, neurotransmitters, growth factors, antigens and inflammatory mediators (Nishizuka, Y. (1988) Nature, 334:661). When these extracellular agents bind to their specific cell surface receptors, they stimulate the hydrolysis of phosphatidylinositol, phosphatidyl choline or phosphatidylethanolamine, resulting in the accumulation of diacylglycerol which, in turn, activates PKC. This activation of PKC causes specific cellular substrates to be phosphorylated, resulting in the regulation of cellular processes which are closely linked to the physiological control of contractile, secretory and proliferative processes (Nishizuka, Y. (1984) Nature, 308:693). Physiological responses induced by the regulatory system in which PKC participates include proto-oncogene activation (Nishizuka, Y. (1986) Science, 233, 305-312), serotonin release from platelets (Kaibuchi, et al. (1982) Cell Calcium, 3:323; Kaibuchi, et al. (1985) J. Biol. Chem., 258:6701), lysosomal enzyme release and superoxide generation from neutrophils (Kajikawa, et al. (1983) Biochem. Biophys. Res. Commun., 116:743; Sehau, et al. (1983) Biochem. Biophys. Acta, 762:420), histamine release from mast cells (Kata Kami, et al. (1982) Biochem. Biophys. Res. Commun., 121:573), secretion of aldosterone from adrenal glomerulus (Kojima, et al. (1983) Biochem. Biophys. Res. Commun., 116:555), and contraction of vascular smooth muscle (Rasmussen, et al. (1984) Biochem. Biophys. Res. Commun., 122:776). Thus, it is known that PKC participates in many

important physiological responses *in vivo*. These physiological responses have been implicated in the onset and progression of a variety of disease states, including cancer, cardiovascular and renal disorders, inflammation, immunosuppression, septic shock and central nervous system disorders. Therefore, an inhibitor of PKC is expected to be useful in the treatment of such diseases.

Recently, we have found that homogentisic acid derivatives, previously shown to inhibit growth of *B. subtilis*, *E. coli*, and *P. atroventum*, are surprisingly also PKC inhibitors and hence are expected to have utility in the treatment of conditions wherein PKC inhibition is indicated.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a compound of Formula (I)



(I)

wherein:

R_1 and R_2 are independently methyl or hydrogen;

R_3, R_4 and R_5 are independently bromide or hydrogen;

and R_6 is hydroxy, methoxy or amino;

excluding a compound of Formula (I) wherein:

R_1 and R_2 are hydrogen;

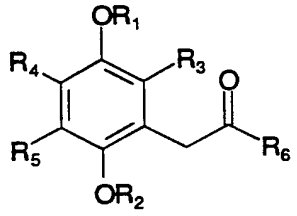
R_3 and R_4 are bromine;

R_5 is hydrogen; and

R_6 is amino;

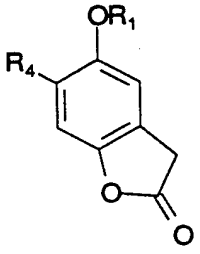
or a pharmaceutically acceptable salt thereof.

In another aspect, the present invention provides a method of treatment of mammals suffering from disease states where inhibition of PKC is therapeutically desirable, e.g., cancer, cardiovascular disorders, renal disorders; inflammation, central nervous system disorders, immunosuppression and septic shock; which method comprises administering to a mammal in need of such treatment an effective amount of a compound selected from the group consisting essentially of a compound of Formula (I):



(I)

a compound of Formula (II):



(II)

wherein:

R_1 and R_2 are independently methyl or hydrogen;

R_3, R_4 and R_5 are independently bromine or hydrogen; and

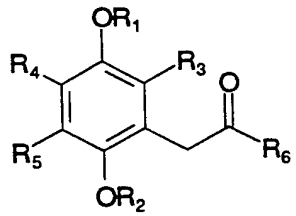
R_6 is methoxy, hydroxy or amino,

or a pharmaceutically acceptable salt thereof.

In still another aspect, the present invention also provides a pharmaceutical composition comprising of a compound of Formula (I), a compound of Formula (II), or a pharmaceutically acceptable salt thereof, useful in the treatment of such diseases wherein inhibition of PKC is indicated.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a compound of Formula (I):



(I)

wherein:

R_1 and R_2 are independently methyl or hydrogen;

R_3, R_4 and R_5 are independently bromide or hydrogen;

and R_6 is hydroxy, methoxy or amino;

excluding a compound of Formula (I) wherein:

R_1 and R_2 are hydrogen;

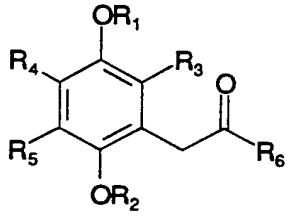
R_3 and R_4 are bromine;

R_5 is hydrogen; and

R_6 is amino;

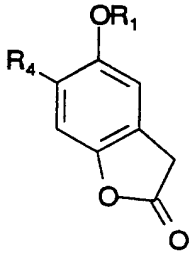
or a pharmaceutically acceptable salt thereof, such compounds having utility as PKC inhibitors.

The present invention also provides a method for treating disease in mammals, including humans, for which inhibition of PKC is indicated. The method comprises administration to a mammal, preferably a human, in need thereof, at least one compound of a class of PKC inhibitors selected from the group consisting essentially of a compound of Formula (I):



(I)

a compound of Formula (II):



(II)

wherein,

R_1 and R_2 are independently methyl or hydrogen;

R_3, R_4 and R_5 are independently bromide or hydrogen; and

R_6 is methoxy, hydroxy or amino,

or a pharmaceutically acceptable salt thereof.

The compounds of Formula (I) and Formula (II) inhibit both the catalytic fragment (PKM) and the intact enzyme of PKC with equal potency, suggesting, not wishing that the present invention be construed as in any way limited by a particular mechanism or mode of action, that these compounds are catalytic domain inhibitors. The compounds of the present invention do not inhibit phorbol ester binding to PKC, a fact which is consistent with activity being at the catalytic rather than the regulatory domain of the enzyme. The present compounds are selective, as demonstrated by the fact that they do not inhibit cAMP-dependent protein kinase (PKA).

Accordingly, the compounds of Formula (I) and Formula (II) of the present invention are useful for the treatment of PKC-mediated disease states, including, without limitation, cancer, as adjuvant therapy for use with antineoplastic compounds to ameliorate or prevent multiple drug resistance (MDR) of the target neoplastic cells to such antineoplastic compounds; cardiovascular diseases, that is, heart and circulatory diseases such as thrombosis, atherosclerosis, arteriosclerosis, ischemia, reperfusion injury, and hypertension, preferably hypertension; immunosuppressive and inflammatory disorders, such as asthma, rheumatoid arthritis, psoriasis, inflammatory bowel disease, preferably psoriasis; central nervous system diseases, such as stroke and trauma; septic shock based on PKC activation; and ischemia-induced renal failure.

The method of treatment of the present invention concerns the use of compounds of Formula (I) and Formula (II) as PKC inhibitors in the treatment of such PKC-mediated disease states.

The compound of Formula (I), wherein R_1 and R_2 are hydrogen, R_3 and R_4 are bromine, R_5 is hydrogen, and R_6 is amino has been isolated from the marine sponge, *Verongia Aurea* (Krejcarek, et al., *Tetrahedron Letters*, **8**, 507-510 (1976)). Similarly, we have discovered that the novel compound of Formula (I), wherein R_1 and R_2 are hydrogen, R_3 and R_4 are bromine, R_5 is hydrogen, and R_6 is methoxy is also extractable from *Verongia Aurea*. Both compounds were isolated by chromatography on silica gel and further purification by preparative thick layer chromatography (ptlc) of a triturated extract prepared from the freeze-dried sponge following extraction with ethyl acetate and methanol.

The semisynthetic dibromo-homogentisic acid analogs of the present invention, wherein R_1 and R_2 are hydrogen, can be conveniently prepared from homogentisic acid methyl ester and homogentisic lactone. The term "homogentisic" means 2,5-dihydroxyphenyl acetic acid. Either 2,5-dihydroxyhomogentisic acid methyl ester or 2,5-dihydroxyphenylacetic-γ-lactone are dissolved in ether and excess bromine is added. The reaction mixture is stirred for several hours, preferably 1 to 24 hours, more preferably 2 to 12 hours, at ambient temperatures. The solution is washed with sodium sulfite followed by sodium carbonate and then extracted with ether. The ether layer is then evaporated and the resulting compound is purified by silica gel ptlc.

More specifically, 3,4,6-tribromo-2,5-dihydrophenyl acetic acid methyl ester (R_1 and R_2 are hydrogen, R_3, R_4 and R_5 are bromine and R_6 is methoxy) and 3,4-dibromo-2,5-dihydroxyphenylacetic acid methyl ester (R_1 and R_2 are hydrogen, R_3 is hydrogen, R_4 and R_5 are bromine and R_6 is methoxy) are prepared by dissolving homogentisic acid methyl ester in ether and adding excess bromine. The reaction mixture is stirred for 3 to 12 hours at room temperature. The solution is washed with sodium sulfite followed by sodium bicarbonate and then extracted with ether. The ether layer is then evaporated and the compounds may be purified by silica gel ptlc.

3-Bromo-2,5-dihydroxyphenylacetic acid methyl ester (R_1, R_2, R_3 and R_5 are hydrogen, R_4 is bromine and R_6 is methoxy) and 4-bromo-2,5-dihydroxyphenylacetic-γ-lactone (R_1 is hydrogen and R_4 is bromine) are prepared by dissolving 2,5-dihydroxyphenylacetic acid methyl ester in ether and adding excess bromine. The reaction mixture is stirred for 2 to 3 hours at room temperature. The solution is washed with sodium sulfite followed by sodium carbonate and then extracted with ether. The ether layer is then evaporated to yield a brown gummy substance. The compounds are then purified by silica gel ptlc.

The monomethoxy derivative of the lactone and the dimethoxy derivative of the methyl ester analogs, wherein R_1 and, where applicable, R_2 are methyl, may be synthesized in the following manner. A solution of one of the above described compounds and dimethyl sulphate in dry acetone containing potassium carbonate is refluxed at about 80°C for about 12 hours. The solvent is evaporated to dryness and the resulting residue is dissolved in water and extracted with chloroform.

Evaporation of the organic layer yields a substance, which when purified by silica gel ptlc, yields the methylated derivative of the starting compound.

More specifically, 4-bromo-2,5-dimethoxyphenylacetic acid methyl ester (R_1 and R_2 are methyl, R_3 and R_5 are hydrogen, R_4 is bromine and R_6 is methoxy), 3,4-dibromo-2,5-dimethoxyphenylacetic acid methyl ester (R_1 and R_2 are methyl, R_3 is hydrogen, R_4 and R_5 are bromine and R_6 is methoxy) and 3,4,6-tribromo-2,5-dimethoxyphenylacetic acid methyl ester (R_1 and R_2 are methyl, R_3, R_4 and R_5 are bromine and R_6 is methoxy) are prepared, respectively, by refluxing a solution containing either 4-bromo-2,5-dihydroxyphenylacetic acid methyl ester, 3,4-dibromo-2,5-dihydroxyphenylacetic acid methyl ester or 3,4,6-tribromo-2,5-dihydroxyphenylacetic acid methyl ester, respectively, with dimethyl sulphate in dry acetone containing potassium carbonate at about 80°C for about 12 hours. The solvent is evaporated to dryness and the resulting residue is dissolved in water and extracted with chloroform. The organic layer is evaporated and the dimethoxy derivative is purified by silica gel ptlc.

4-Bromo-5-methoxyphenylacetic-γ-lactone (R_1 is methyl and R_4 is bromine) is prepared under the same conditions as described above, however, the starting material is 4-bromo-5-hydroxyphenylacetic-γ-lactone.

A pharmaceutical composition of the present invention comprises one or more compounds of Formulae (I) or (II), or a pharmaceutically acceptable salt thereof, in any pharmaceutically acceptable combination, together with an appropriate pharmaceutical carrier or excipient. Appropriate pharmaceutical carriers may be either solid or liquid. The administration of such a composition may be parenteral, rectal, topical, transdermal or oral, preferably oral. Pharmaceutical forms include, but are not limited to, syrups, suspensions or emulsions, tablets, capsules and lozenges.

A liquid formulation will generally consist of a suspension or solution of the compound or pharmaceutically acceptable salt thereof in a suitable liquid carrier. Suitable liquid carriers include, but are not limited to, ethanol, glycerin, non-aqueous solvents such as polyethylene glycol, oils, or water with a suspending agent, preservatives, flavorings, or coloring agents, or any suitable combination thereof.

A composition in the form of a tablet can be prepared using any suitable pharmaceutical carrier routinely used for preparing solid formulations. Examples of

such carriers include, but are not limited to, magnesium stearate, starch, lactose, sucrose and cellulose.

A composition in the form of a capsule can be prepared using routine encapsulation procedures. For example, pellets, granules or powder containing a compound of Formula (I) or Formula (II), can be prepared using standard carriers and then filled into a hard gelatin capsule. Alternatively, a dispersion or suspension can be prepared using any suitable pharmaceutical carrier and the dispersion or suspension is then filled into a soft gelatin capsule. Suitable pharmaceutical carriers include aqueous gums, celluloses, silicates and oils.

A composition for parenteral administration can be formulated as a solution or suspension. Said solution or suspension will generally consist of a compound of Formula (I) or Formula (II) in a sterile aqueous carrier or parenterally acceptable oil. Examples of parenterally acceptable oils include, but are not limited to, polyethylene glycol, polyvinyl pyrrolidone, lecithin, arachis oils and sesame oil. Alternatively, the solution can be lyophilized and then reconstituted with a suitable solvent just prior to administration.

The pharmaceutical preparations of the present invention are made using conventional techniques of the pharmaceutical chemist and involve mixing, granulating, and compressing, when necessary, for tablet forms, or mixing, filling and dissolving the ingredients, as appropriate, to give the desired oral, parenteral, rectal, transdermal, or topical products.

Preferably the composition is in unit dose form. Each dosage unit for parenteral or oral administration contains preferably from 100 mg to 1000 mg total of one or more of a compound of Formula (I), a compound of Formula (II), or a pharmaceutically acceptable salt thereof.

The daily dosage regimen for a subject in need of PKC inhibition may be, for example, an intravenous, subcutaneous, or intramuscular dose of between 100 mg and 1000 mg total of one or more of a compound of Formula (I), a compound of Formula (II), or a pharmaceutically acceptable salt thereof, in any pharmaceutically acceptable combination, the compound being administered 1 to 4 times per day. Suitable compounds will be administered for a period of continuous therapy. Dosages for oral administration may be higher.

No unexpected toxicological effects are expected when compounds of the invention are administered in accordance with the present invention.

BIOLOGICAL DATA

Using the compounds of the present invention, wherein R_1 and R_2 are hydrogen, R_3 and R_4 are bromine, R_5 is hydrogen and R_6 is methoxy or amino, designated as dibromohomogentisic acid methyl ester and dibromohomogentisic acid amide, respectively, the following tests were performed:

1. *In Vitro* Enzyme Assays:

A. Rat brain (partially purified protein kinase C)

Purification of Protein Kinase C from Rat Brain

Protein kinase C is purified from rat brain following the procedure of Walton et al. (Walton, G.H., Bertics, P.J., Hudson, L.G., Vedvick, T.S., and Gill, G.N., Anal. Biochem. 161:425-437 (1987)) and Woodget and Hunter (Woodget, J.R. and Hunter, T., J. Biol. Chem. 268:4836-4843 (1987)) with the following modifications. Ammonium sulfate precipitation is performed twice (first time to 33% saturation, and the second to 70%). After centrifugation, pellets are resuspended and desalted using a G-100 column. Peak fractions are pooled, brought to 16% glycerol and 0.01% Triton X-100, and frozen in small aliquots.

Screening for Protein Kinase C Inhibitors

A high throughput screening assay utilizing 96-well microtiter plates has been developed to identify potential inhibitors of protein kinase C. The incubation volume in each well is 50 microliters containing 10 mM Tris, pH 7.5, 1.1 mM $CaCl_2$; 10 mM $MgCl_2$; 1.0 mM EGTA, 40 micrograms/ml phosphatidyl serine, 1 microgram/ml Diolein; and 100 micrograms/ml histone or other suitable substrate. The reaction is initiated by addition of 0.5 microcuries of γ-^{32}P-ATP (10 micromolar final concentration) subsequent to addition of the various concentrations of test compounds or extracts. The reaction is stopped after 10 minutes at 37°C by spotting 25 microliters of the reaction mixture onto Whatman P81 paper squares using a multichannel pipettor. The squares are washed extensively in 0.5% phosphoric acid, dried with acetone, and assayed for radioactivity by liquid scintillation spectrometry. The concentrations of ATP, histone, and phosphatidyl

serine used in the assay permit identification of inhibitors of both catalytic and regulatory sites of protein kinase C.

In the above assay, the IC_{50} for inhibition of PKC for dibromohomogentisic acid methyl ester was approximately 500 nM. The IC_{50} for dibromogentisic acid amide was approximately 500 nM.

B. Inhibitory Effect on *in vitro* TNF production by human monocytes

Assay Set-up

The effects of compounds of Formula (I) on the *in vitro* production of TNF by human monocytes is examined using the following protocol.

Human peripheral blood monocytes are isolated and purified from either blood bank buffy coats or plateletpheresis residues, according to the procedure of Collota, R. et al., J. Immunol., 132(2):936 (1984).

TNF Assay:

Immunoassay plates (96-well, Immunolon 4) are coated for 2 hours at room temperature with a murine anti-human TNFα antibody (16 μg/ml in 50 mM PBS, pH 7.5). Plates are blocked with PBS containing 0.5% casein, 0.01% thimersol, 0.001% phenol red and 0.25% Tween-20 (Block buffer) for 1 hour at 37ºC. After three washings with wash buffer (consisting of PBS and 0.05% Tween-20), test samples are added to the plate and incubated overnight at 4°C. A standard titration curve is obtained making serial dilutions of a known sample of recombinant human TNFα in block buffer identical to the test samples. Next, the plates are washed five times with wash buffer and incubated with rabbit ant-human antibody (1:1000 dilution) in block buffer for 2 hours at 37°C. Plates were washed five times with wash buffer and incubated with peroxide conjugated goat anti-rabbit antibody (1:5000 in block buffer) for 2 hours at 37°C. Following five more washes with wash buffer, substrate (o-phenylenediamine in 0.1M citrate buffer, pH 4.5 containing 0.1% urea peroxide) is added to the plates for 20 min, and the color reaction is stopped by addition of 0.1 M sodium fluoride. Spectroscopy (462 nM) is performed using a microELISA autoreader (Titertek Multiscan MC).

The compounds of Formula (I) blocked LPS-induced TNF release from human monocytes.

C. *In Vitro* Determination of Phorbol Ester Binding

Tritiated phorbol myristate acetate (^{3}H-PMA) is added to membrane fractions containing PKC. Membrane fractions are prepared by homogenizing rat kidney medulla in 50 mM Tris buffer, pH 7.5, containing 5 mM EGTA, 10 mM dithiothreitol, 1 mM PMSF, 1 mg/ml bacitracin and 5 mM leupeptin at 4°C. The homogenate is centrifuged at 200,000 x g for 30 minutes. The pellet is resuspended in the above buffer and used as the membrane fraction. Incubation mixture containing 20 mM Tris maleate, pH 6.8, 1 mM EGTA, 100 mM KCl, 1.1 mM $CaCl_2$, 100 micrograms/ml phosphatidylserine; 50 micrograms of membrane fraction and test compounds in various concentrations are prepared. The phorbol ester binding is initiated by the addition of saturating concentration (20 nM) of [^{3}H]phorbol 12,13 dibutyrate. The binding is terminated after 30 minutes by the addition of ice cold dimethyl sulfoxide. Free and bound ligand are separated by filtering through Whatman GF/B filters presoaked in 0.3% polyethyleneimine using a Brandel cell harvester. The filters are washed with cold DMSO, placed in scintillation vials with scintillant, and counted. The amount of radioactivity bound to the membrane versus the amount of radioactivity free in the supernatant is then compared. Table I presents typical test results, further test results are presented with the Examples. The concentration of test compound which inhibits 50% of the binding is the IC_{50}.

Compound	IC_{50} (μM)
4,6-dibromohomogentisic acid methyl ester	1.0
4,6-dibromohomogentisic acid amide	1.0

Table I

D. Reversal of MDR Phenotype of Colchicine -Resistant Chinese Hamster Ovary (Cultured) Cells

Chinese hamster ovary cells, line CH^RC5, have the MDR phenotype and are somewhat cross-resistant to topotecan, presumably because of P-glycoprotein (Pgp)-mediated efflux of the compound from the cells (Hendricks, C.B., et al. (1992) *Cancer Res.* 52: 2268-2278). This protein has been shown to be activated by PKC.

Treatment of CH^RC5 cells with PKC inhibitors for 2h prior to the addition of topotecan reverses their MDR phenotype, as judged by increased cellular activity of topotecan. This activity is assayed as the production of DNA strand breakage. CH^RC5 cells normally sustain small amounts of DNA strand breakage when incubated with 10 μM topotecan for 30 minutes at 37°C. If the cells are incubated in 1-2 μM 4,6-dibromohomogentisic acid methyl ester or 4,6-dibromohomogentisic acid amide for 2h prior to topotecan treatment, twice as much DNA damage is produced by 10 μM topotecan than in the absence of these PKC inhibitors. 1-2 μM is the IC_{50} range for this effect of 4,6-dibromohomogentisic acid methyl ester and 4,6-dibromohomogentisic acid amide.

EXAMPLES

The following Examples are purely illustrative and are provided to teach how to make and use the compounds of the present invention, but are not intended to limit the scope of the present invention in any manner.

EXAMPLE 1

Preparation of 3,4,6-tribromo-2,5-dihydroxyphenylacetic acid methyl ester

2,5-Dihydroxyhomogentisic acid methyl ester (100 mg) was dissolved in ether (50 ml) and to that solution was added excess bromine (2 ml). The reaction mixture was stirred for about12 hours at room temperature. The resulting reddish solution was washed with sodium sulfite to remove excess bromine followed by sodium carbonate to neutralize the HBr formed in the reaction product. The solution was then extracted with ether. The ether layer was evaporated and the product (58 mg) was then purified by silica gel ptlc. The compound was identified from its spectral data. ^{1}H-NMR (MeOH-d_4): δ3.94 (s, 2H), 3.68 (s, 3H); MS: *m/z* 416 ($C_9H_7Br_3O_4$); PKC activity >600 μM.

EXAMPLE 2

Preparation of 3-bromo-2,5-dihydroxyphenylacetic acid methyl ester

2,5-Dihydroyphenylacetic-γ-lactone (100 mg) was dissolved in ether (50 ml) and to that was added excess bromine (2 ml). The reaction mixture was stirred for about 2 hours at room temperature. The solution was washed with sodium sulfite to

remove excess bromine followed by sodium carbonate to neutralize the HBr formed in the reaction product. The solution was then extracted with ether. The ether layer was evaporated to yield a brown gum which after silica gel ptlc afforded 3-bromo-2,5-dihydrophenylacetic acid methyl ester (74 mg). The compound was identified from its spectral data ^{1}H-NMR (MeOH-d_4): δ7.19 (s, 1H), 6.85 (s, 1H);3.93 (s, 2H), 3.68 (s, 3H); MS: *m/z* 260 ($C_9H_9BrO_4$); PKC activity = 18 μM.

EXAMPLE 3

Preparation of 3,4-dibromo-2,5-dihydroxyphenylacetic acid methyl ester

2,5-Dihydroxyphenylacetic acid methyl ester (100 mg) was dissolved in ether (50 ml) and to that solution was added excess bromine (2 ml). The reaction mixture was stirred for about 3 hours at room temperature. The solution was washed with sodium sulfite to remove excess bromine followed by sodium carbonate to neutralize the HBr formed in the reaction product. The solution was then extracted with ether and the ether layer was evaporated to give a colorless powder (76 mg).. This powder was purified by silica gel to afford pure 3,4-dibromohomogentisic acid methyl ester and identified from its spectral data. ^{1}H-NMR (MeOH-d_4): δ6.77 (s, 1H), 3.67 (s, 3H), 3.61 (s, 2H); MS: *m/z* 338 ($C_9H_8Br_2O_4$); PKC activity = 0.38 μM.

EXAMPLE 4

Preparation of 4-bromo-2,5-dihydroxyphenylacetic-γ-lactone

2,5-Dihydroxyphenylacetic-γ-lactone (50 mg) was dissolved in ether (25 ml) and to that was added excess bromine (1 ml). The reaction mixture was stirred for 3 hours at room temperature. The reddish solution The solution was washed with sodium sulfiteto remove excess bromine followed by sodium carbonate to neutralize the HBr formed in the reaction product. The solution was then extracted with ether. The ether layer after evaporation and subsequent purification by silica ptlc afforded 4-bromo-2,5-dihydrophenylacetic-γ-lactone (18 mg) which was identified from its spectral data ^{1}H-NMR (MeOH-d_4): δ7.20 (s, 1H), 6.88 (s, 1H), 3.35 (s, 2H); MS: *m/z* 228 ($C_8H_5BrO_3$); PKC activity = 21 μM.

EXAMPLE 5

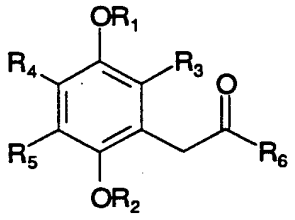
Preparation of 3,4,6-tribromo-2,5-dimethoxyphenylacetic acid methyl ester

A solution of 3,4,6-tribromo-2,5-dihydroxyphenylacetic acid methyl ester (25 mg) and dimethyl sulphate (0.2 ml) in dry acetone (50 ml) containing potassium carbonate was refluxed at about 80°C for about 12 hours. The solvent was evaporated to dryness. The residue was dissolved in water (how much?) and extracted with chloroform (how much?). Evaporation of the organic layer resulted in a colorless gum which, when purified by silica gel ptlc, yielded 3,4,6-tribromo-2,5-dimethoxyphenylacetic acid methyl ester (26 mg) as an amorphous powder. The compound was identified by NMR and mass spectra. ^{1}H-NMR (MeOH-d_4): δ3.95 (s, 2H), 3.89 (s, 3H), 3.81 (s, 1H); MS: *m/z* 444 ($C_{11}H_{11}Br_3O_4$); PKC activity > 100 μM.

The above description and Examples fully disclose the present invention and preferred embodiments thereof, including how to make and use the present invention. However, it is understood that the present invention is not limited to the particular embodiments described herein above, but includes all modifications thereof within the scope of the following claims.

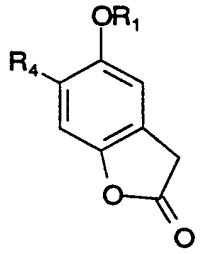
We claim:

1. A method of treatment for PKC-mediated disease states comprising administering to a mammal in need thereof a compound selected from a group consisting of a compound of Formula (I):



(I)

a compound of Formula (II):



(II)

wherein,

R_1 and R_2 are independently methyl or hydrogen;

R_3, R_4 and R_5 are independently bromide or hydrogen; and

R_6 is methoxy, hydroxy or amino,

or a pharmaceutically acceptable salt thereof.

2. A method according to claim 1 wherein said compound is a compound of Formula (I).

3. A method according to claim 2 wherein said compound is a compound of Formula (I) wherein:

R_1 and R_2 are hydrogen;

R_3 and R_4 are bromine;

R_5 is hydrogen; and

R_6 is methoxy or amino.

4. A method according to claim 1 wherein said disease state is a cardiovascular disorder.

5. A method according to claim 5 wherein said cardiovascular disorder is hypertension.

6. A method according to claim 1 wherein said disease state is inflammation.

7. A method according to claim 6 wherein said inflammation causes arthritis.

8. A method according to claim 1 wherein said disease state is cancer.

9. A method according to claim 1 wherein said compound is used for adjuvant therapy together with antineoplastic compounds to abrogate or prevent multiple drug resistance in treatment of cancer.

10. A method according to claim 1 wherein said disease state is renal failure.

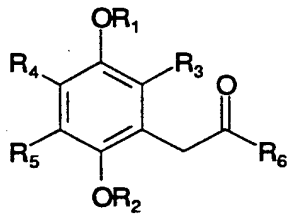
11. A method according to claim 1 wherein said disease state is septic shock.

12. A method according to claim 1 wherein said disease state is an immunosuppressive disorder.

13. A method according to claim 1 wherein said disease state is a central nervous system disorder.

14. A method according to claim 12 wherein said immunosuppressive disorder is psoriasis.

15. A compound of Formula (I):



(I)

wherein:

R_1 and R_2 are independently methyl or hydrogen;

R_3, R_4 and R_5 are independently bromide or hydrogen;

and R_6 is hydroxy, methoxy or amino;

excluding a compound of Formula (I) wherein:

R_1 and R_2 are hydrogen;

R_3 and R_4 are bromine;

R_5 is hydrogen; and

R_6 is amino;

or a pharmaceutically acceptable salt thereof.

16. A compound according to claim 15 wherein:

R_1 and R_2 are hydrogen;

R_3 and R_4 are bromine;

R_5 is hydrogen; and

R_6 is methoxy.

17. The compound according to claim 15 wherein:

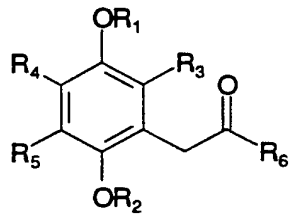
R_1 and R_2 are hydrogen;

R_3 is hydrogen;

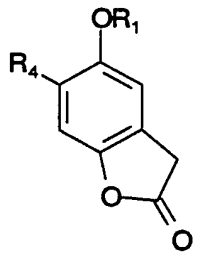
R_4 and R_5 are bromine; and

R_6 is methoxy.

18. A pharmaceutical composition comprising a compound selected from a group consisting essentially of a compound of Formula (I):



and a compound of Formula (II):



wherein:

R_1 and R_2 are independently methyl or hydrogen;

R_3, R_4 and R_5 are independently bromide or hydrogen; and

R_6 is methoxy, hydroxy or amide,

or a pharmaceutically acceptable salt thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/03489

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07C 69/76, 65/00, 65/01, 23/00; C07D 307/00, 307/78; A01N 43/08
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 560/055, 075; 562/465, 478; 564/170; 549/305, 310; 514/469, 532, 570, 622

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN CAS structure search; file CA; file MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Tetrahedron, Volume 44, No. 15 issued 1988. M. Norte et al. "Aplysinadiene and (R,R)-5-[3,5-dibromo-4-[(2-oxo-5-oxazolidinyl)] Methoxy-Phenyl]-2-oxazolidinone, Two novel Metabolites from Aplysina Aerophoba. Synthesis of Aplysinadiene" see pages 4973-498, especially page 4975, compounds 23 and 24.	15-18
X	Chemical Abstracts, Vol. 98, issued 1982, Maruyama et al., "Acrid components of vegetable foods. I acrid components of bamboo shoot" Abstract No. 196689, Kaseigaku Kenbyu (1982) 29 (1) 1-5.	15-18

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 16 JUNE 1995	Date of mailing of the international search report 18 JUL 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PAULA K. KILLOS aco Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/03489

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Chemical Abstracts, Volume 116, issued 1991, Dai, J et al., "Studies of Indonesian medicinal plants, Part 3. Phenylacetic acid Derivative and a Thioamide glycoside from Entada Phaseolides" Abstract No. 91201, Phytochemistry (1991), 30 (11), 3749-52.	1, 2, 8, 9
E, X	WO, A, 95/08335 (Zanetti) 30 March 1995 entire reference, particularly page 25.	1-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/03489

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

560/055, 075; 562/465, 478; 564/170; 549/305, 310; 514/469, 532, 570, 622